UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

April 25, 2015

MEMORANDUM

Subject: Protocol Review for 777PA2; DB Barcode: D425823.

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Applicant: Reckitt Benckiser Inc.
Morris Corporate Center IV
399 Interpace Parkway
Parsippany, NJ 07054

I. BACKGROUND

Reckitt Benckiser (RB) intends to determine efficacy of product following the EPA OCSPP Guideline 810.2500. Through the current submission, the registrant is resubmitting a new efficacy protocol for air sanitization entitled “RB Protocol to Assess Reduction in Bacterial Contamination”. Protocol was developed by Reckitt Benckiser, LLC located at 99 Interpace Parkway, Parsippany, NJ 07054.

This data package identified as D425823 contained a letter from the applicant’s representative (dated January 30, 2015), two studies (MRID nos. 495629-01 and 497427-01).

II. BRIEF DESCRIPTION OF THE PROTOCOL

Note: During the course of the protocol review, the version 1 (dated January 30, 2015,) has be replaced with the version 2 (dated October 1, 2015) with MRID 497427-01. The following is the review of version 2 of the protocol.

Title: RB PROTOCOL TO ASSESS REDUCTION IN BACTERIAL CONTAMINATION

Purpose:
The purpose of this study is to evaluate the ability of a test substance to provide a temporary reduction in the number of bacteria in an aerosol chamber to support air sanitization labeling claims.

Method Reference:
ASTM International (2013). Annual Book of Standards. Standard Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporicidal Activities of Chemicals. Document #E2197. ASTM, Barr Harbor Drive, West Conshohocken, PA 1942.
Centers for Disease Control and Prevention (2009). *Biosafety in Microbiological and Biomedical Laboratories*, 5th Edition,Publication No. 21-1112.
Environmental Protection Agency (2013) – Air Sanitizers - Efficacy Data Recommendations). Test Guideline No. #OCSPP 810.2500-Air Sanitizers-2013-03-12 [EPA 730-C-11-003] (http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0150-0025)
Miles A.A., Misra S.S. (1938). The estimation of the bactericidal power of the blood. *J. Hyg.* **38**: 732–749.
Organization for Economic Cooperation and Development (2013). Guidance Document on Quantitative Methods for Evaluating the Activity of Microbicides used on Hard Non-Porous Surfaces. OECD document No. ENV/JM/MONO(2013)11. OECD, Paris, France.
Springthorpe, V.S. and Sattar, S.A. (2007). Application of a quantitative carrier test to evaluate microbicides against mycobacteria. J. AOAC International 90:817-824.

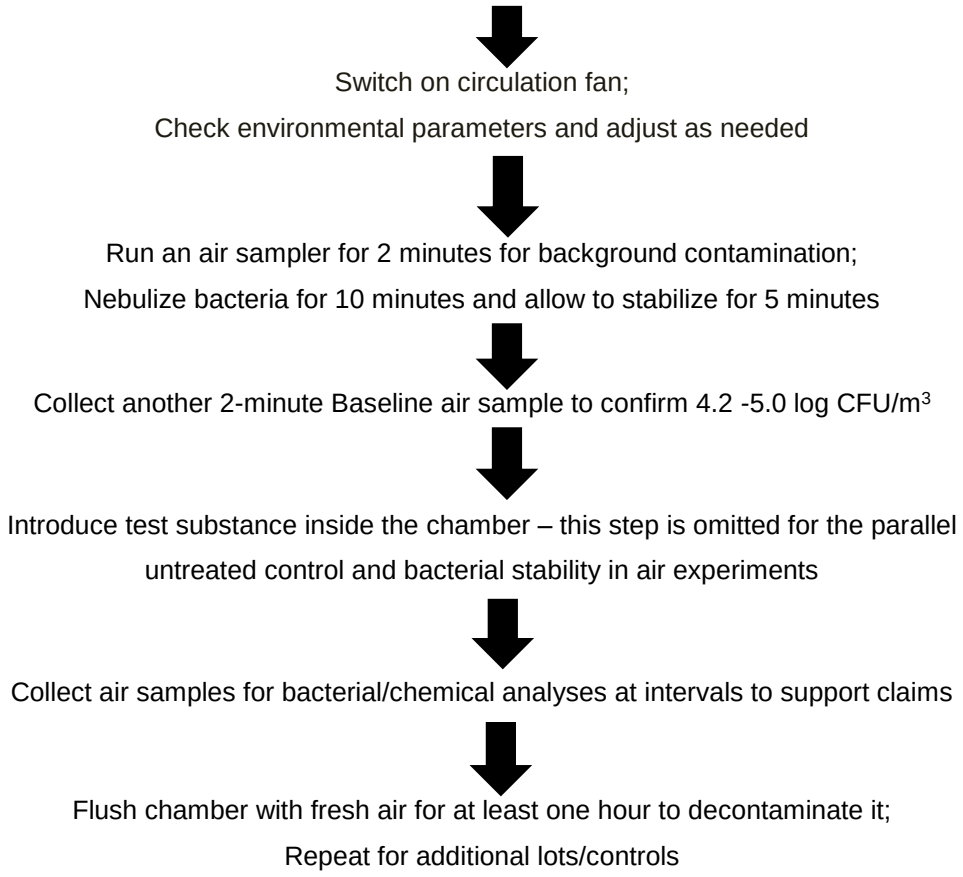
Test System (Microorganism):
Staphylococcus aureus (ATCC 6538)
Klebsiella pneumoniae (ATCC 4352)
Pseudomonas aeruginosa (ATCC 15442)
Additional Bacteria (e.g.*, Acinetobacter baumannii*)

Procedure:

B**asic design of the aerobiology chamber:**

- The aerosol chamber (Figure 1) is an enclosure with a volume of 24.00 M^3 located inside a biosafety level-3 (BSL-3) facility with controlled access. Polyethylene sheeting (0.006 inches/0.1524 mm thick) is affixed to a steel-framed structure with polyvinyl chloride (PVC) adhesive tape to represent the walls, ceiling and floor to maintain an airtight seal. Sealable ports, window and door provide access to the inside of the chamber for maintenance and to place and remove any monitoring devices to be used. The plastic sheeting can be easily and safely removed, decontaminated as biohazardous waste by autoclaving and discarded when no longer required. The sheeting is grounded with a copper wire to dissipate any static electricity that may accumulate. Similarly, the copper wire used to suspend the PVC air sampling pipe acts as grounding wire.
- In accordance with the current EPA guidelines (2012), the chamber does not permit any air exchanges; nor does it contain any furniture or fixtures in accordance with EPA 810.2500 study design description. Furniture and fixtures were not placed in the chamber inside of the BSL facility due to biosafety and decontamination concerns over the multiple test dates over a long period.
- To assess the airborne survival of the test bacteria or to determine the activity of any air sanitization technology, the air in the chamber is sampled at the rate of 28.3 L/minute using an externally-placed slit-to-agar air (STA) sampler with a built-in vacuum pump (e.g., Particle Measuring Systems, Boulder, CO; Model 790020-1000). This programmable device can be set to operate for a minimum air sampling time of 2 minutes to as long as five hours, and the actual length of sample collection time will be determined by the anticipated load of viable bacteria in the air of the chamber. The air exiting the sampler is discharged directly into the BSL-3 facility's HEPA-filtered exhaust system. For the baseline value, the concentration of the test bacteria in the nebulizer fluid should be adjusted to achieve a minimum of 4.2 log10 to a maximum of 5.0 log10 CFU per M3 at the start of the treatment.
- A built-in lever-activated port (Figure 1) allows a container with the chemical(s) to be attached to the chamber and the container's trigger pressed for the required length of time to release the test substance into the chamber. The start and stop times (clock times) will be recorded for the application of the treatment to the air. The official exposure period or contact time begins upon completion of the release of the test substance which should begin after the nebulizer has completed the 10-minute release of the test bacteria, five minutes for stabilization of the aerosols and the 2 minute pre-treatment air sample is taken.
- Any formulation can also be placed inside the chamber and activated from the outside or by accessing it with the gloves affixed to the chamber (Figure 1). The labeled use directions will be based upon the test substance application procedure used during testing.
- The exposure period (contact time) may vary with the Test Substance. The same exposure period will be used to evaluate each lot of a Test Substance and controls. The air will be sampled for the same duration and at the same intervals for each lot of a Test Substance and Controls but no fewer than three air samplings per lot per microorganism will be collected.

Experimental Design: A generic sequence of the main steps in the operation of the chamber is given in the Flowchart below.



Study Acceptance Criteria:

- Test Substance Performance Criteria: After correction for bacterial settling and natural biological decay, the test substance must demonstrate ≥99.9% (3 $\log_{10}$) reduction in the viability of the bacterial species over the parallel untreated control.
- Baseline Acceptance Criteria: The control recovery must demonstrate a minimum of 4.2 $\log_{10}$ to a maximum of 5.0 $\log_{10}$ CFU/m^3 at the start of the treatment for a valid test.

Control Acceptance Criteria:

- All sterility controls must be free of any visible growth.
- Viability Control must demonstrate growth in all media with <100 CFU/plate.
- Purity Control must demonstrate a pure culture.
- Neutralization Validation: The mean number of CFU on the plate unexposed to the test substance and those on the plate exposed to the test substance must be within 20%.
- Magnehelic readings must indicate no leaks in the chamber during an experiment.
- Temperature and RH readings must stay within range required for the test.

Retesting Guidance

For tests where the product passes and the mean Baseline value is above 5.0 $\log_{10}$ CFU/m^3, no retesting is necessary. For tests where the product fails and the mean *Baseline* is above

III. CONCLUSION

1. The submitted protocol (MRID 497427-01) **is adequate** for assessing the reduction of air bacterial concentration.

2. As air sanitizing guidance 810.2500 stated, contact times of 5 minutes or less are to be considered for Air Sanitization.

3. The proposed control bacterial air concentrations of 4.2 $\log_{10}$ to a maximum of 5.0 $\log_{10}$ CFU/m^3 put the claims on relatively clean to moderately clean room air concentrations such as healthy office building and house rooms (excluding farm and agricultural premises).

4. For consistency on amount of product delivered in the air, the registrant must use "Total Release Fogger" products.

5. For treatment of rooms equipped with HVAC system, returns and registers must be closed or sealed (and shut air system down if possible).

6. It is a reminder that product lots must be tested at the LCL. The lowest effective air treatment concentration must be used. Air treatment concentration may be set in conjunction with lower certified limit of active ingredient concentration in Total Release Fogger can (TRF). See following table for examples of consideration where all Xs may be equal (X1=X2=X3=X4= X).

Volume ft^3 (m^3)	**Effective Air Concentration X** (Gram/m^3)	**Product to Use** (or units of TRF to use)
0 (0.0) to 353.15 (10.0)	X1	1
353.15 (10.0) to 706.3 (20.0)	X2	2
706.3 (20.7) to 1059.45 (30.0)	X3	3
1059.45 (30.0) to 1412.6 (40.0)	X4	4

7. In addition to the AD Efficacy Team, this protocol was also evaluated by an independent peer review panel. The peer review panel addressed specific questions posed by AD regarding the protocol methods and test applicability. The panel's findings were considered in the final conclusions of this review.